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LEAF SURFACE CHEMICALS FROM *Nicotiana* AFFECTING GERMINATION OF *Peronospora tabacina* (ADAM) SPORANGIA

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Abstract—A bioassay was used to evaluate the effects of cuticular leaf components, isolated from *N. tabacum*, *N. glutinosa* (accessions 24 and 24a), and 23 other *Nicotiana* species, on germination of *P. tabacina* (blue mold). The leaf surface compounds included α- and β-4,8,13,-duvatriene-1,3-diols (DVT-diols), (13-*E*)-labda-13-ene-8α-,15-diol (labdenediol), (12-*Z*)-labda-12,14-diene-8α-ol (*cis*-abienol), (13-*R*)-labda-8,14-diene-13-ol (manool), 2-hydroxymanool, a mixture of (13-*R*)-labda-14-ene-8α,13-diol (sclareol), and (13-*S*)-labda-14-ene-8α,13-diol (episclareol), and various glucose and/or sucrose ester isolates. The above in acetone were applied onto leaf disks of the blue mold-susceptible *N. tabacum* cv. TI 1406, which was then inoculated with blue mold sporangia. Estimated IC_{50} values (inhibitory concentration) were 3.0 μg/cm^2 for α-DVT-diol, 2.9 μg/cm^2 for β-DVT-diol, 0.4 μg/cm^2 for labdenediol and 4.7 μg/cm^2 for the sclareol mixture. Manool, 2-hydroxymanool, and *cis*-abienol at application rates up to 30 μg/cm^2 had little or no effect on sporangium germination. Glucose and/or sucrose ester isolates from the cuticular leaf extracts of 23 *Nicotiana* species and three different fractions from

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N. bigelovii were also evaluated for antimicrobial activity at a concentration of 30 $\mu g/cm^2$. Germination was inhibited by >20% when exposed to sugar esters isolated from *N. acuminata, N. benthamiana, N. attenuata, N. clevelandii*, and *N. miersii*, and accessions 10 and 12 of *N. bigelovii*. These results imply that a number of compounds may influence resistance to blue mold in tobacco.

Key Words—*Nicotiana*, *Peronospora tabacina*, blue mold, leaf surface chemistry, diterpenes, sugar esters.

INTRODUCTION

The leaf surfaces of most higher plants have trichomes, which produce natural secretions that serve as mitigating factors in herbivory and disease development in some plant species (Behnke, 1984). In some tobacco (*Nicotiana tabacum* L.) and *Nicotiana* species, chemical compounds found in trichome exudates affect host plant resistance to several insect pests (Severson et al., 1985, 1991), but their role in disease resistance in tobacco is less clear. Removal of the leaf surface chemicals by washing the leaves with water or acetone increased the plants' susceptibility to *Peronospora tabacina* Adam (Hill, 1966; Reuveni et al., 1987), the obligate fungal pathogen responsible for tobacco blue mold, a disease of major economic importance (Main, 1991). In other studies, specific trichome exudate constituents have been reported to have fungicidal properties against germination of *P. tabacina* sporangia (Bailey et al., 1974; Cruickshank et al., 1977; Cutler et al., 1986; Lawson et al., 1988). These constituents include the diterpenes α- and β-4,8,13-duvatriene-1,3,-diols (DVT-diols), and the labdane, (12-*Z*)-labda-12,14-dien-8α-ol, which are synthesized in the gland cells of tobacco trichomes (Keene and Wagner, 1985; Kandra and Wagner, 1987).

In in vitro experiments, Menetrez et al. (1990) found that germination of *P. tabacina* sporangia was completely inhibited when exposed to 10 $\mu g/cm^2$ of the DVT-diols and was stimulated at much lower concentrations. Inhibition of germination of *P. tabacina* sporangia by *cis*-abienol was also reported, but this compound was less active than the DVT-diols (Menetrez et al., 1990). Sucrose esters, another exudate constituent common to many tobacco genotypes (Severson et al., 1991), reportedly had no effect on *P. tabacina* sporangium germination (Menetrez et al., 1990). The sucrose ester fraction tested in that experiment was isolated from a single tobacco genotype. It is possible that sucrose esters isolated from other genotypes may have antimicrobial activity as genetic variation for sugar ester type, and ester moiety composition does exist among *N. tabacum* genotypes and *Nicotiana* species (Severson et al., 1991). The objective of our research was to determine the effects of certain trichome exudate components on *P. tabacina* sporangium germination using an in vivo system. This included further characterization of the fungicidal activity of the

DVT-diols and *cis*-abienol and testing of additional exudate compounds, including several labdanes and a number of sugar esters isolated from *Nicotiana* species.

METHODS AND MATERIALS

Plants. Seed of Tobacco Introduction (TI) 1406 were germinated in a commercial soil mix in the greenhouse. Plants were transferred into plastic pots (8.5 cm diameter) containing steam-sterilized soil mixture (soil–sand–peat, 2:2:1 v/v/v) and then moved to a growth chamber kept at 24°C with a 12-hr photoperiod of fluorescent light. Plants were fertilized twice a week with Peters 20:20:20 (N/P/K) water-soluble fertilizer.

Isolation and Purification of Leaf Surface Compounds. Cuticular components were extracted from *N. tabacum* cultivars NC 2326 and NFT, *N. glutinosa* (accessions 24 and 24a), and 24 other *Nicotiana* species grown in field plots at Georgia Coastal Plain Experiment Station, Tifton, Georgia, and the Crops Research Laboratory, Oxford, North Carolina. Tops cut from plants six to eight weeks after transplantation were extracted with methylene chloride (Baker Resi-analyzed reagent grade) as previously described (Jackson et al., 1986; Severson et al., 1988). *cis*-Abienol (from NFT), α-DVT-diol, and β-DVT-diol (from NC 2326) were isolated in 98+% purity as described by Severson et al. (1988). The (13-*E*)-labda-13-ene-8α,15-diol (labdenediol) (from *N. glutinosa* no. 24a) and the sclareols, a mixture of (13-*R*)- and (13-*S*)-labda-14-ene-8α,13-diol (sclareol and episclareol), and (13-*R*)-labda-8,14-diene-2α,13-diol (2-hydroxymanool) (from *N. glutinosa* no. 24) were obtained in 98+% purity as described by Jackson et al. (1986). (13-*R*)-labda-8,14-diene-13-ol (manool) was obtained from Aldrich Chemical Co.

The cuticular extracts from the other *Nicotiana* species in Table 1 were taken to dryness on a rotoevaporator, and the residue was partitioned between hexane and 80% methanol–water as described by Severson et al. (1988). The methanol–water-soluble fraction (100–200 μg) containing the sugar esters was dissolved in 0.5 ml of 90% methanol–water, loaded into an injection loop, and chromatographed on a Bio-Sil C-18 (HL Silica, fully endcapped; 40–63 μm; Bio-Rad Laboratories) preparative reverse phase column (1.25 cm ID × 15 cm). The column was eluted with 225 ml 60% methanol–water, 90 ml 90% methanol–water, and 125 ml 100% methanol, and 5-ml fractions were collected. The solvent was removed on a Savant SpeedVac and fractions with observable residue were analyzed by capillary gas chromatography (Severson et al., 1984; Jackson et al., 1986). Fractions containing glucose and/or sucrose esters were combined to yield the sugar ester isolates tested. The chromatography cleanly separated the glucose and sucrose ester fractions in the *N. attenuata* sample, and they were combined separately.

TABLE 1. EFFECT OF GLUCOSE AND/OR SUCROSE ESTERS PARTITIONED FROM LEAF WASHES OF DIFFERENT *Nicotiana* SPECIES (ACCESSIONS) ON *P. tabacina* SPORANGIUM GERMINATION

Treatment (30 $\mu g/cm^2$)	Spores (N)[a]	Inhibition (%)
Experiment 1		
Control	110.8 ± 28.6	(62.4)[b]
N. noctiflora	96.7 ± 23.1	−0.6
N. nudicaulis	93.3 ± 22.6	3.4
N. pauciflora	115.7 ± 30.8	3.7
N. acuminata	105.9 ± 20.0	23.3[c]
N. miersii	101.1 ± 22.0	33.9[c]
N. clevelandii	102.5 ± 24.6	39.8[c]
Experiment 2		
Control	157.0 ± 35.5	(72.6)
N. langsdorfii	155.6 ± 39.3	4.6
N. palmerii	177.1 ± 45.4	5.9[c]
N. velutina[d]	168.9 ± 39.6	12.7[c]
N. rustica	172.0 ± 43.2	14.2[c]
N. bonariensis	173.4 ± 27.4	15.8[c]
N. maritima[d]	179.3 ± 30.8	16.3[c]
Experiment 3		
Control	51.3 ± 16.7	(31.0)
N. cavicola[d]	45.2 ± 9.0	−13.5
N. gossei	45.3 ± 10.5	−9.0
N. simulans[d]	45.8 ± 13.7	−6.0
N. plumbaginifolia	53.6 ± 20.6	−0.6
N. occidentalis	46.4 ± 11.5	4.7
N. bigelovii (13)	52.9 ± 17.3	13.2
Experiment 4		
Control	119.1 ± 37.3	(39.6)
N. hesperis	137.9 ± 16.7	−12.0
N. umbratica	105.6 ± 22.9	6.1
N. debneyi	116.7 ± 25.2	7.1
N. amplexicaulis	108.4 ± 28.6	10.3
N. benthamiana	113.2 ± 32.7	20.5
N. bigelovii (12)	108.4 ± 14.6	23.9[c]
N. attenuata	114.4 ± 29.9	27.9[c]
N. bigelovii (10)	116.1 ± 29.3	33.2[c]

[a] Data are the means ± standard deviation of 10 replications.
[b] Figures in parentheses are the mean percent germination.
[c] Means do not differ significantly from control treatment ($P < 0.05$) by paired t test.
[d] Blue mold-resistant species (Rufty et al., 1990).

Inoculum Production. P. tabacina inoculum was maintained following a procedure adapted by Cohen and Kuć (1980). Six-week-old plants of the blue mold-susceptible tobacco cultivar KY 14 were sprayed to runoff with a fresh sporangial suspension (2×10^5 sp/ml) of *P. tabacina* isolate KY-79. Plants were held at 22–24°C in a dark chamber with 100% relative humidity for 18 hr. Plants were then transferred to a growth chamber with 75% relative humidity and a 12-hr photoperiod for disease development. Sporangium formation was induced when chlorotic lesions developed (seven to eight days after inoculation) by placing plants in darkness for 24 hr at 20°C and 100% relative humidity. Fresh inoculum for each experiment was obtained by lightly brushing the sporulating leaf surface with cold sterile distilled water. To remove any germination inhibitors, sporangium suspensions were filtered and rinsed thoroughly using a 3.0-μm Millipore filter system. Sporangia were resuspended in sterile distilled water, and sporangium concentration was calculated with the aid of a hemacytometer.

Bioassay. Leaf disks (50 mm^2) excised from interveinal areas of leaves from 5- to 6-week-old TI 1406 plants were placed adaxial side up on wet filter paper in a Petri dish. A stock solution of each leaf surface compound was prepared by dissolving each compound in acetone. Chemical concentrations were prepared by diluting portions of the stock solution with acetone. A 10-μl aliquot of each treatment solution was applied evenly to the entire surface of each leaf disk using an Eppendorf repeater pipet. Leaf disks were then set aside to dry prior to inoculation. The following control treatments were included in each experiment: (1) no chemical or acetone was applied and (2) an acetone blank in which there was no leaf surface chemical.

Using an airbrush sprayer (Paashe Airbrush Co., Harwood Heights, Illinois), leaf disks were uniformly inoculated with a 500-μl sporangium suspension of *P. tabacina* (10×10^4–15×10^4 sp/ml) at 50 psi. To maintain a high humidity, the inner surface of the Petri dish covers were sprayed with distilled water. Plates were incubated for 24 hr at 15°C in the dark. Leaf disks in each plate were then sprayed with 500 μl of a 0.01% solution of the fluorescent stain CalcoFluor White MR2 (Polysciences, Inc., Warrington, Pennsylvania), removed from the filter paper, and arranged on glass slides. Slides were fixed by applying drops of ACCU-Mount 60 Mounting Medium (Stephens Scientific, Denville, New Jersey) and topped with cover slips. Sporangium germination was evaluated by fluorescence microscopy differentiation as developed by Wigglesworth et al. (1990). The study included a series of separate experiments. First, those chemicals reported previously to have antimicrobial activity were tested using a dilution series. Second, sclareol, labdenediol, manool, and 2-hydroxymanool were tested at a relatively high concentration. A dilution series experiment was performed for any of these compounds that displayed activity at the high concentration. Third, glucose and/or sugar ester mixtures from 24

Nicotiana species were tested at a concentration of 30 $\mu g/cm^2$. A completely randomized design was used for each experiment with 10 replications per treatment.

Analysis. Percent inhibition was calculated individually for each experiment by using the following formula: % inhibition = [(% germination of control treatment − % germination at each concentration tested)/% germination of control treatment] × 100. The log dose (concentration)–response relationship for each of the treatments was linearized using probits and inhibitory concentrations (IC_{50}) were estimated (SAS Institute Inc., 1985).

RESULTS

The in vivo bioassay was used to approximate the natural infection process and to permit observation of germ tube growth and infection plug formation. An integral part of this bioassay is the leaf tissue to which we applied the test chemicals and inoculum of the obligate pathogen, *P. tabacina*. We selected the blue mold-susceptible TI 1406 to provide the leaf tissue for the bioassay because this genotype is classified as having nonsecreting glandular trichomes, and it lacks the trichome exudate mass typically found on other tobacco genotypes such as those from which the test chemicals were extracted. To further reduce any possible complicating exudate compounds that may be produced by TI 1406, we used leaf tissue from juvenile plants grown under fluorescent lighting (Severson et al., 1985). Young plants produce little exudate and synthesis of trichome secretory product is low for plants grown under fluorescent lamps. Although TI 1406 has only minimal exudate compounds under optimum growth conditions, it does have cuticular waxes. Capillary gas chromatograms of the leaf surface washes of TI 1406 and several genotypes that have secreting glandular trichomes have similar profiles except for those compounds found in the trichome exudate (Nielsen and Severson, 1990). Thus, TI 1406 would likely have few if any leaf surface compounds that could influence detection of the activity of the test compounds.

Microscopic evaluation using fluorescence revealed that sporangia and germ tubes fluoresced bright blue against a red leaf surface background; thus, germination assessments were readily attainable. Sites of germ tube penetration of the leaf were apparent, and in some cases appressoria formation was visible. No morphological differences were observed among sporangia, and germ tube length did not appear to be affected when exposed to any of the chemical or control treatments. In all experiments, the acetone control had little or no effect on sporangium germination when compared to the untreated leaf disks, and thus only the acetone control was used for comparison purposes. A different batch of fresh inoculum was used for each experiment, and this resulted in some

variation in sporangium germination from one experiment to the next. Germination of sporangia for the control treatments was from 31% to 70% among the different experiments with a mean of 49%. Data from these different experiments were not compared directly as these experiments were performed separately.

IC_{50} values were 3.0 $\mu g/cm^2$ for α-DVT-diol and 2.9 $\mu g/cm^2$ for β-DVT-diol (Table 2). A similar inhibitory response was observed when sporangia were exposed to the α- + β-DVT-diol mixture. Percent inhibition after exposure of sporangia to α- + β-DVT-diol at the highest concentration tested was similar to inhibition levels of sporangia when exposed to the individual isomers at the same concentration. Sporangia were not completely inhibited from germinating at concentrations up to 120 $\mu g/cm^2$ of α-DVT-diol or β-DVT-diol. However, sporangium germination was reduced nearly 80% at DVT-diol concentrations of 30 $\mu g/cm^2$. There appeared to be little additional effect of increasing DVT-diol beyond 30 $\mu g/cm^2$. Furthermore, at very low concentrations of DVT-diols, including the mixture of the two isomers, there was no indication of any consistent stimulatory effect of these chemicals on sporangium germination.

The next leaf surface chemical tested was *cis*-abienol, which also had been reported previously to affect *P. tabacina* sporangium germination. In a dilution series experiment (data not shown), *cis*-abienol was tested for inhibitory effects at concentrations ranging from 3.75×10^{-4} $\mu g/cm^2$ to 120 $\mu g/cm^2$. Sporangium germination was not greatly reduced when exposed to any of the 10 concentrations of *cis*-abienol when compared to the control treatment. At the two lowest concentrations of *cis*-abienol (3.75×10^{-4} and 3.75×10^{-3} $\mu g/cm^2$), sporangium germination was greater than for the acetone control treatment.

Sclareols, manool, labdenediol, and 2-hydroxymanool, were initially tested at a single concentration of 30 $\mu g/cm^2$ to determine if any of these inhibited *P. tabacina* sporangium germination. Manool appeared to have no activity at this concentration. Although 2-hydroxymanool significantly reduced sporangium germination, the magnitude of the reduction was much less than that observed for the sclareol mixture and labdenediol. Thus, dilution series experiments were conducted only for the latter two chemicals.

Labdenediol and the sclareol mixture had IC_{50} values of 0.4 $\mu g/cm^2$ and 4.7 $\mu g/cm^2$, respectively (Table 3). Percent inhibition after exposure to additional amounts of the sclareol mixture (>15 $\mu g/cm^2$) was similar to inhibition levels of sporangia when exposed to 15 $\mu g/cm^2$. A similar response in sporangium germination was observed among concentrations of labdenediol ranging from 3.75 $\mu g/cm^2$ to 30 $\mu g/cm^2$. However, 60 $\mu g/cm^2$ of labdenediol caused nearly a 90% reduction in sporangium germination.

A mixture of DVT-diols, labdenediol, and sclareols were tested to determine if there was a synergistic effect among these compounds. The chemicals were mixed in a 1 : 1 : 1 ratio at all the concentrations. The ratio of the α and β isomers of DVT-diol was 2 : 1 within the DVT-diol component of the mixture.

TABLE 2. PERCENT INHIBITION OF *P. tabacina* SPORANGIA AFTER EXPOSURE TO α-DVT-DIOL, β-DVT-DIOL, AND α- + β-DVT-DIOL MIXTURE IN SEPARATE EXPERIMENTS

Concentration ($\mu g/cm^2$)	α-DVT-diol		β-DVT-diol		α- + β-DVT-diol	
	Spores (N)[a]	Inhibition (%)	Spores (N)	Inhibition (%)	Spores (N)	Inhibition (%)
Control	187.4 ± 31.1	(50.7)[b]	88.2 ± 19.2	(35.4)	136.3 ± 27.8	(70.2)
3.75×10^{-4}	184.6 ± 25.4	2.1	93.1 ± 22.0	21.5	154.0 ± 47.6	4.6
3.75×10^{-3}	183.7 ± 26.5	−5.3	88.5 ± 23.4	−3.9	141.4 ± 30.3	−0.7
3.75×10^{-2}	184.7 ± 15.5	1.2	94.9 ± 30.0	1.8	140.6 ± 33.9	1.9
3.75×10^{-1}	172.3 ± 37.7	11.8	96.3 ± 23.4	28.3	143.2 ± 35.6	3.2
3.75	188.4 ± 29.7	59.0	90.4 ± 20.7	50.3	132.7 ± 38.0	52.9
7.5	174.8 ± 15.0	68.8	84.8 ± 20.0	56.2	172.2 ± 78.8	59.8
15.0	166.8 ± 19.6	78.6	83.2 ± 16.2	68.3	127.3 ± 37.0	65.6
30.0	176.3 ± 23.6	78.5	83.7 ± 22.0	81.4	132.4 ± 46.1	78.1
60.0	163.1 ± 20.5	83.1	76.3 ± 21.9	81.8	116.4 ± 42.8	79.8
120.0	165.2 ± 20.4	85.7	79.6 ± 18.2	83.7	103.0 ± 14.3	82.3
IC_{50}[c]		3.0		2.9		5.0

[a] Data are the means ± standard deviation of 10 replications.
[b] Figures in parentheses are the mean percent germination.
[c] Inhibitory concentration ($\mu g/cm^2$) estimated by probit analysis.

TABLE 3. PERCENT INHIBITION OF *P. tabacina* SPORANGIA AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF LABDENDIOL AND SCLAREOL MIXTURE

Concentration ($\mu g/cm^2$)	Labdenediol		Sclareols	
	Spores (N)[a]	Inhibition (%)	Spores (N)	Inhibition (%)
Control	176.0 ± 48.0	(38.5)[b]	183.9 ± 61.5	(41.8)
9.38×10^{-1}	171.6 ± 31.7	47.5	210.2 ± 64.0	22.2
1.88	192.6 ± 33.8	63.0	211.5 ± 65.9	36.4
3.75	193.2 ± 35.1	76.8	222.3 ± 55.0	56.0
7.5	204.5 ± 47.8	78.2	193.8 ± 62.2	57.0
15.0	187.4 ± 48.9	74.0	202.3 ± 83.4	65.1
30.0	180.5 ± 63.5	77.7	194.2 ± 50.7	67.1
60.0	180.0 ± 57.8	89.3	178.3 ± 54.5	67.2
IC_{50}[c]		0.4		4.7

[a] Data are the means ± standard deviation of 10 replications.
[b] Figures in parentheses are the mean percent germination.
[c] Inhibitory concentration ($\mu g/cm^2$) estimated by probit analysis.

TABLE 4. PERCENT INHIBITION OF *P. tabacina* SPORANGIA AFTER EXPOSURE TO MIXTURE OF α- + β-DVT-DIOLS, LABDENEDIOL, AND SCLAREOLS

Concentration ($\mu g/cm^2$)	Mixture[a]	
	Spores (N)[b]	Inhibition (%)
Control	220.4 ± 57.8	(50.7)[c]
4.69×10^{-1}	207.6 ± 54.8	11.6
9.38×10^{-1}	227.5 ± 67.6	21.4
1.88	212.9 ± 46.3	28.4
3.75	221.4 ± 67.2	67.0
7.5	193.7 ± 70.3	46.5
15.0	171.1 ± 41.1	71.4
30.0	192.9 ± 53.6	82.7
IC_{50}[d]		4.2

[a] Compound mixture of (2/3 : 1/3) : 1 : 1 (α- + β-DVT-diols)–labdenediol–sclareols at all concentrations.
[b] Data are the means ± standard deviation of 10 replications.
[c] Figures in parentheses are the mean percent germination.
[d] Inhibitory concentration ($\mu g/cm^2$) estimated by probit analysis.

Sporangium germination was not inhibited completely following exposure to the mixture at any concentrations tested. The IC_{50} value was 4.2 $\mu g/cm^2$ for this mixture (Table 4).

Sugar ester mixtures from 23 *Nicotiana* species and three different fractions from *N. bideglovii* were tested for antimicrobial activity at a single concentration of 30 $\mu g/cm^2$. Compared to the control treatments, leaf washes from five of the species, *N. acuminata*, *N. attenuata*, *N. benthamiana*, *N. clevelandii*, and *N. miersii*, and accessions 10 and 12 of *N. bigelovii* inhibited sporangium germination by > 20% (Table 1).

DISCUSSION

The data presented provide further support for the hypothesis that certain tobacco leaf surface components may influence resistance to blue mold in tobacco. Menetrez et al. (1990) observed in in vitro experiments total inhibition by the DVT-diols at 10 $\mu g/cm^2$ and a stimulation in germination as the DVT-diols were diluted.

Using an in vivo bioassay, we found that although sporangium germination was never inhibited completely, a 78% reduction in germination was observed when sporangia were exposed to 30 $\mu g/cm^2$ of the individual DVT-diol isomers or an isomer mixture. The concentrations at which we found α- and β-DVT diols inhibited sporangium germination were well within those levels reported to be found on leaves of field-grown plants of *N. tabacum* (Severson et al. 1985). IC_{50} values of 5.0 $\mu g/cm^2$ for the DVT-diols were less than is often applied for commercial fungicides. Cruickshank et al. (1977) reported the β isomer of the DVT-diols was more inhibitory than the α isomer, and recently Menetrez et al. (1990) suggested that the α isomer was more toxic. No relative differences were observed between the individual isomers in separate experiments of our study. On tobacco leaves these compounds are often found together as part of the exudate mass in a 2:1 ratio of α and β isomers, and therefore their individual activity may be of minor importance.

Menetrez et al. (1990) applied *cis*-abienol to the surface of water agar and found that *P. tabacina* sporangium germination was significantly inhibited. However, they found *cis*-abienol activity was suppressed when it was combined with other compounds including hydrocarbons and sucrose esters, which are also found in the leaf surface. We did not find *cis*-abienol to have any inhibitory activity at any of the concentrations tested. Possible interactions between *cis*-abienol and other leaf surface components not produced by trichomes may explain the lack of biological activity from *cis*-abienol in this in vivo system. Moreover, purified *cis*-abienol in acetone readily recrystallizes upon solvent reduction, and

crystalline masses on the leaf surface may not interact with the blue mold sporangium.

Sclareol, a leaf surface diterpene isolated from *N. glutinosa*, also has been reported to have biological activity. Bailey et al. (1974) exposed a wide range of fungal colonies and sporangia to sclareol and found that sclareol did not affect germination, but it did inhibit mycelial development on agar media. We found that when sporangia were exposed to 3.75 $\mu g/cm^2$ of a mixture of sclareol and episclareol, germination was inhibited by 56%. We believe this is the first report to find that sclareol inhibits sporangium germination.

In dilution series experiments, labdenediol, having a IC_{50} value of 0.4 $\mu g/cm^2$, was the most active leaf surface component tested. Higher concentrations of labdenediol further reduced sporangium germination, and at the highest concentration tested germination was nearly 90% inhibited.

Cutler et al. (1986) found sucrose esters inhibited the growth of gram-positive bacteria such as *Bacillus subtilis* but were not effective against the growth of gram-negative bacteria and the fungi *Curvularia lunata* and *Aspergillus flavus*. *P. tabacina* sporangia were not affected by sucrose esters in in vitro experiments (Menetrez et al., 1990). In our experiments, sugar esters from the different *Nicotiana* species showed less antimicrobial activity than the terpenoid compounds. Germination was reduced from 20% to 40% by the different sugar esters from six *Nicotiana* species, none of which have been reported to have blue mold resistance. This anomaly may have several explanations. Among these are that the sugar esters exist with a matrix of other compounds in the trichome exudate and their antimicrobial activity may be obscured by this matrix in evaluations for blue mold resistance. Additionally, tests identifying the relative resistance of *Nicotiana* species to blue mold may not have been conducted under plant growth conditions suitable for optimal biosynthesis of exudate compounds. These glucose and sucrose esters vary with the type of acid substitution on the glucose or sucrose moieties and most often consist of mixtures of these different esterified compounds. Because these sugar esters make up a small portion of the leaf surface profile, their individual significance in blue mold resistance needs further investigation.

In addition to genetic background, other factors have been reported to influence the leaf surface composition, including age of development and agronomic and environmental factors (Severson et al., 1985). While fungi-toxic leaf surface chemicals may not provide complete resistance to blue mold, we believe that they may have a significant role in the epidemiology of the disease. Elucidation of inheritance patterns for specific exudate constituents and trichome traits in tobacco permits plant breeders and geneticists to manipulate the composition and concentration of trichome exudate (Nielsen and Severson, 1990). This capability, coupled with the knowledge of antimicrobial or insecticidal

activity of exudate compounds, could enable the development of genotypes with enhanced pest resistance.

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